Attorney's Docket No.: 07265-191001 / DM-3575

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Isaacs et al.
Serial No. : 09/627,600
Filed : July 28, 2000
Title : ACTIVATION OF PEPTIDE PRODRUGS BY HK2

Art Unit : 1653
Examiner : H. Robinson

BOX SEQUENCE
Commissioner for Patents
Washington, D.C. 20231

RESPONSE TO NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE AND/OR AMINO ACID SEQUENCES

In response to the communication dated September 21, 2001 (copy enclosed), applicants submit herewith a Sequence Listing in computer readable form as required by 37 CFR §1.824. In addition, applicants submit an initial Sequence Listing as required under 37 CFR §1.823(a) and a statement under 37 CFR §1.821(f).

Applicants respectfully request entry of the paper copy and computer readable copy of the Sequence Listing filed herewith for the instant application. Furthermore, applicants request entry of the following amendments.

In the specification:

Insert the paper copy of the Sequence Listing filed herewith following the Oath/Declaration.

Replace the paragraph beginning at page 4, line 10, with the following rewritten paragraph:

--Fig. 1 is a portion of the amino acid sequence of Semenogelin I (SEQ ID NOs:1-4) and Semenogelin II (SEQ ID NOs:5-11), showing the cleavage sites for human kallikrein 2.--

CERTIFICATE OF MAILING BY FIRST CLASS MAIL

I hereby certify under 37 CFR §1.8(a) that this correspondence is being deposited with the United States Postal Service as first class mail with sufficient postage on the date indicated below and is addressed to the Commissioner for Patents, Washington, D.C. 20231.

October 19, 2001
Date of Deposit

Shaheen Mehjabeen
Signature

SHAHEEN MEHJABEEN
Typed or Printed Name of Person Signing Certificate

Replace the paragraph beginning at page 5, line 29, with the following rewritten paragraph:

--Some examples of preferred peptides include (Note that the symbol][denotes an hK2 cleavage site):

1. Lys-Arg-Arg][(SEQ ID NO:12)
2. Ser-Arg-Arg][(SEQ ID NO:13)
3. Ala-Arg-Arg][(SEQ ID NO:14)
4. His-Arg-Arg][(SEQ ID NO:15)
5. Gln-Arg-Arg][(SEQ ID NO:16)
6. Ala-Phe-Arg][(SEQ ID NO:17)
7. Ala-Gln-Arg][(SEQ ID NO:18)
8. Ala-Lys-Arg][(SEQ ID NO:19)
9. Ala-Arg-Lys][(SEQ ID NO:20)
10. Ala-His-Arg][(SEQ ID NO:21)--

Replace the paragraph beginning at page 6, line 12, with the following rewritten paragraph:

--Additional preferred peptides of longer sequence length include:

11. Gln-Lys-Arg-Arg][(SEQ ID NO:22)
12. Lys-Ser-Arg-Arg][(SEQ ID NO:23)
13. Ala-Lys-Arg-Arg][(SEQ ID NO:24)
14. Lys-Lys-Arg-Arg][(SEQ ID NO:25)
15. His-Lys-Arg-Arg][(SEQ ID NO:26)
16. Lys-Ala-Phe-Arg][(SEQ ID NO:27)
17. Lys-Ala-Gln-Arg][(SEQ ID NO:28)
18. Lys-Ala-Lys-Arg][(SEQ ID NO:29)
19. Lys-Ala-Arg-Lys][(SEQ ID NO:30)
20. Lys-Ala-His-Arg][(SEQ ID NO:31)--

Replace the paragraph beginning at page 6, line 24, with the following rewritten paragraph:

--Additional preferred peptides that include an X-1 amino acid are:

21. Lys-Arg-Arg][Leu (SEQ ID NO:32)
22. Ser-Arg-Arg][Leu (SEQ ID NO:33)
23. Ala-Arg-Arg][Leu (SEQ ID NO:34)
24. Ala-Arg-Arg][Ser (SEQ ID NO:35)
25. His-Arg-Arg][Ala (SEQ ID NO:36)
26. Gln-Arg-Arg][Leu (SEQ ID NO:37)
27. Ala-Phe-Arg][Leu (SEQ ID NO:38)
28. Ala-Gln-Arg][Leu (SEQ ID NO:39)
29. Ala-Lys-Arg][Leu (SEQ ID NO:40)
30. Ala-Arg-Lys][Leu (SEQ ID NO:41)
31. Ala-His-Arg][Leu (SEQ ID NO:42)--

Replace the paragraph beginning at page 7, line 7, with the following rewritten paragraph:

--Preferred peptides of still longer sequence length having X_{-1} include:

32. His-Ala-Gln-Lys-Arg-Arg][Leu (SEQ ID NO:43)
33. Gly-Gly-Lys-Ser-Arg-Arg][Leu (SEQ ID NO:44)
34. His-Glu-Gln-Lys-Arg-Arg][Leu (SEQ ID NO:45)
35. His-Glu-Ala-Lys-Arg-Arg][Leu (SEQ ID NO:46)
36. Gly-Gly-Gln-Lys-Arg-Arg][Leu (SEQ ID NO:47)
37. His-Glu-Gln-Lys-Arg-Arg][Ala (SEQ ID NO:48)
38. Gly-Gly-Ala-Lys-Arg-Arg][Leu (SEQ ID NO:49)
39. His-Glu-Gln-Lys-Arg-Arg][Ser (SEQ ID NO:50
40. Gly-Gly-Lys-Lys-Arg-Arg][Leu (SEQ ID NO:51)
41. Gly-Gly-His-Lys-Arg-Arg][Leu (SEQ ID NO:52)--

Replace the paragraph beginning at page 15, line 28, with the following rewritten paragraph:

--Recombinant hK2 was produced and purified as described in Lövgren et al., *Biochem. Bioph. Res. Co.*, 238, 549-555 (1997). Semenogelin I and II were isolated from human semen as described previously in Malm et al., *Eur. J. Biochem.*, 238, 48-53 (1996). The tripeptide aminomethylcoumarin (AMC) substrates Boc-Phe-Ser-Arg-AMC (SEQ ID NO:53), Boc-Gln-Gly-Arg-AMC (SEQ ID NO:54), H-Pro-Phe-Arg-AMC (SEQ ID NO:55), boc-Val-Pro-Arg-AMC (SEQ ID NO:56), H-D-Val-Leu-Lys-AMC (SEQ ID NO:57), Tos-Gly-Pro-Arg-AMC- (SEQ ID NO:58), Tos-Gly-Pro-Lys-AMC (SEQ ID NO:59), Z-Leu-Leu-Arg-AMC (SEQ ID NO:60), Z-Val-Val-Arg-AMC (SEQ ID NO:61), Z-Ala-Arg-Arg-AMC (SEQ ID NO:62), and H-Arg-Gln-Arg-Arg-AMC (SEQ ID NO:63) were from Bachem (Bubendorf, Switzerland). The heptapeptide substrates Mu-Ala-Pro-Val-Leu-Ile-Leu-Ser-Arg-AMC (SEQ ID NO:64) and Mu-Val-Pro-Leu-Ile-Gln-Ser-Arg-AMC (SEQ ID NO:65) corresponding to the pro peptides of PSA hK2 were from Enzyme Systems Product (Livermore, CA, USA). ACT was purified from human blood plasma as described in Christenssson et al., *Eur. J. Biochem.*, 194, 755-63 (1990). PCI was provided by Prof. Johan Stenflo (Malmö University Hospital, Malmö, Sweden), and SLPI, and PSTI by Prof. Kjell Ohlsson (Malmö University Hospital, Malmö, Sweden). Benzamidine hydrochloride was from Amresco® (Solon, OH, USA), leupeptin and antipain were from ICN Biomedicals (Costa Mesa, CA, USA), Aprotinin was from Sigma (St. Louis, MO, USA), and PPACK from Calbiochem (La Jolla, CA, USA).--

Replace the paragraph beginning at page 18, line 10, with the following rewritten paragraph:

--Substrates ending in either arginine or lysine were tested. The kinetic constants for hydrolysis of the substrates by hK2 are shown in Table 1. The best substrate was the kallikrein substrate Pro-Phe-Arg-AMC (SEQ ID NO:55) having the highest kcat and k_{cat}/K_m values. The cathepsin B substrate Ala-Arg-Arg-AMC (SEQ ID NO:62) was also cleaved quite effectively having a relatively high k_{cat} value and a low K_m resulting in a four times lower k_{cat}/K_m value than that obtained for the kallidrein substrate Pro-Phe-Arg-AMC (SEQ ID NO:55). However, no

hydrolysis of Arg-Gln-Arg-Arg-AMC (SEQ ID NO:63) was detected. HK2 cleaved additionally Val-Pro-Arg-AMC (SEQ ID NO:56), and Leu-Leu-Arg-AMC (SEQ ID NO:60), but with lower efficiency. As with the semenogelins hK2 also here cleaves substrates with Arg at position P1 and preferentially a large residue or another Arg at position P2. None of the substrates with lysine in the C-terminal position were cleaved.--

Replace Table 1 beginning at page 19, line 1, with the following rewritten table:

--Table 1. Substrate Hydrolysis by hK2

Substrates	Km (M)	Kcat (min^{-1})	Kcat/km ($\mu M^{-1}min^{-1}$).	Activity (%)
Pro Phe Arg-AMC (SEQ ID NO:55)	40	55	1.375	100
Val Pro Arg-AMC (SEQ ID NO:56)	48	1.6	0.034	6
Gly Pro Arg-AMC (SEQ ID NO:58)		NR		
Gly Pro Lys-AMC (SEQ ID NO:59)		NR		
Leu Leu Arg-AMC (SEQ ID NO:60)	71	2.4	0.034	7
Val Val Arg-AMC (SEQ ID NO:61)		NR		
Val Leu Lys-AMC (SEQ ID NO:57)		NR		
Phe Ser Arg-AMC (SEQ ID NO:53)		NR		
Gln Gly Arg-AMC (SEQ ID NO:54)		NR		
Ala Arg Arg-AMC (SEQ ID NO:62)	20	7.2	0.360	33
Arg Gln Arg Arg-AMC (SEQ ID NO:63)		NR		

Replace the paragraph beginning at page 19, line 3, with the following rewritten paragraph:

--The activity listed in Table 1 is the hydrolytic activity of hK2 with 100 μM substrate in relation to the hydrolytic activity of hK2 with 100 μM of the tissue kallikrein substrate H-Pro-Phe-Arg-AMC (SEQ ID NO:55). The entry "N.R." means that no reaction was detected.--

Replace the paragraph beginning at page 19, line 8, with the following rewritten paragraph:

--Activity of hK2 (1.6 pmol) was monitored using the substrate H-Pro-Phe-Arg-AMC (SEQ ID NO:55) (90 μM). Inhibitors, at commonly used concentrations, and hK2 (8.3 nM) were mixed and proteolysis of 90 μM H-Pro-Phe-Arg-AMC (SEQ ID NO:55) was followed up to 20 minutes, starting directly or 10 minutes after mixing the enzyme with various inhibitors. Inhibition was evaluated by comparison with enzyme-free controls.--

Replace the paragraph beginning at page 22, line 27, with the following rewritten paragraph:

--The progress of the reaction of hK2 (8nM final concentration) with the substrate Pro-Phe-Arg-AMC (SEQ ID NO:55) was monitored at two different substrate concentrations without or with different concentrations of PCI (80, 40 or 16 nM final concentration). The fluorescence measurements were started directly after mixing the enzyme with the inhibitor. The inhibitor of hK2 by PCI could be described by the slow-binding inhibition mechanism presented in Scheme 2, which has been used in analyzing the interaction of PCI with various serine proteases (Hermans et al., *Biochem. J.*, 295, 239-245 (1993), and Hermans et al., *Biochemistry*, 33, 5440-44 (1994)). This mechanism assumes that a reversible complex is formed between the proteinase and serine proteinase inhibitor (serpin). The issues justifying the use of the slow binding inhibition mechanism despite the commonly held view that the seprin-proteinase complex is irreversible has been discussed in more detail by Hermans et al. (1993).--

Replace Table 4 beginning at page 25, line 1, with the following rewritten table:

--Table 4. Hydrolysis of hK2 Substrates

Peptide Sequence									hK2 Hydrolysis	Serum Hydrolysis
P7	P6	P5	P4	P3	P2	P1	P'1		Rate (FU/hr/mg)	Rate FU/hr
G	H	E	Q	K	R	R	L	(SEQ ID NO:66)	5966.31	0.17
G	G	G	K	A	R	R	L	(SEQ ID NO:67)	4784.22	0.03
G	G	G	K	A	H	R	L	(SEQ ID NO:68)	4100.94	0.09
G	P	A	H	Q	R	R	L	(SEQ ID NO:69)	4017.81	0.10
G	S	K	G	H	F	R	L	(SEQ ID NO:70)	3029.27	0.04
G	S	K	G	H	R	R	L	(SEQ ID NO:71)	2649.96	UD
G	K	D	V	S	R	R	L	(SEQ ID NO:72)	2316.12	0.08
G	S	Q	N	Q	R	R	L	(SEQ ID NO:73)	2100.48	0.05
G	S	Y	P	S	R	R	L	(SEQ ID NO:74)	2060.21	0.09
G	S	Y	P	S	S	R	L	(SEQ ID NO:75)	1456.18	0.06
G	H	E	Q	K	G	R	L	(SEQ ID NO:76)	650.80	0.04
G	S	N	T	E	R	R	L	(SEQ ID NO:77)	592.34	UD
G	S	Y	E	E	R	R	L	(SEQ ID NO:78)	324.75	0.04
G	K	D	V	S	G	R	L	(SEQ ID NO:79)	242.91	0.05
G	S	N	T	E	K	R	L	(SEQ ID NO:80)	255.90	0.13
G	S	K	G	H	F	H	L	(SEQ ID NO:81)	171.47	0.10
G	S	Q	N	Q	V	R	L	(SEQ ID NO:82)	193.55	0.03
G	P	L	I	L	S	R	L	(SEQ ID NO:83)	118.21	0.07
G	S	Y	E	E	R	H	L	(SEQ ID NO:84)	42.87	0.09
G	K	D	V	S	G	H	L	(SEQ ID NO:85)	67.55	0.05
G	G	G	K	A	H	H	L	(SEQ ID NO:86)	70.15	0.05
G	S	N	T	E	K	H	L	(SEQ ID NO:87)	80.54	0.03
G	P	A	H	Q	D	R	L	(SEQ ID NO:88)	75.34	0.06
G	H	E	Q	K	G	H	L	(SEQ ID NO:89)	1.30	UD
G	P	A	H	Q	D	H	L	(SEQ ID NO:90)	48.06	0.00
G	S	Y	P	S	S	H	L	(SEQ ID NO:91)	24.68	UD
G	S	Q	N	Q	V	H	L	(SEQ ID NO:92)	32.48	0.03--

Replace Table 5 beginning at page 26, line 1, with the following rewritten table:

--Table 5. Additional hK2 Substrates

Substrate Sequence									hK2 Hydrolysis	Serum Hydrolysis
P7	P6	P5	P4	P3	P2	P1	P'1		Rate(FU/hr/mg)	Rate FU/hr
G	H	A	Q	K	R	R	L	(SEQ ID NO:93)	3665.1	0.08
	G	G	K	S	R	R	L	(SEQ ID NO:94)	3439.7	0.03
G	H	E	Q	K	R	R	L	(SEQ ID NO:66)	3366.5	UD
G	H	E	A	K	R	R	L	(SEQ ID NO:95)	3324.1	UD
	G	G	Q	K	R	R	L	(SEQ ID NO:96)	3267.4	0.02
G	H	E	Q	K	R	R	A	(SEQ ID NO:97)	3051.5	0.06
	G	G	A	K	R	R	L	(SEQ ID NO:98)	2773.0	0.02
G	H	E	Q	K	R	R	S	(SEQ ID NO:99)	2638.5	UD
	G	G	K	K	R	R	L	(SEQ ID NO:100)	2583.0	UD
	G	G	H	K	R	R	L	(SEQ ID NO:101)	2428.4	UD
	G	G	K	A	F	R	L	(SEQ ID NO:102)	2374.2	0.07
G	A	E	Q	K	R	R	L	(SEQ ID NO:103)	2325.8	0.10
	G	G	K	A	Q	R	L	(SEQ ID NO:104)	2233.7	0.04
	G	G	K	A	R	R	L	(SEQ ID NO:105)	2171.2	UD
	G	G	K	Q	R	R	L	(SEQ ID NO:106)	2171.2	0.02
	G	G	K	H	R	R	L	(SEQ ID NO:107)	2079.2	UD
G	H	E	Q	A	R	R	L	(SEQ ID NO:108)	1956.4	0.14
	G	G	K	A	K	R	L	(SEQ ID NO:109)	1788.9	0.14
G	H	E	Q	K	R	R	dL	(SEQ ID NO:110)	1690.9	0.15
	G	G	K	A	R	R	S	(SEQ ID NO:111)	1609.5	UD
	G	G	K	A	R	K	L	(SEQ ID NO:112)	1602.4	UD
G	H	E	Q	K	R	R	E	(SEQ ID NO:113)	1473.8	UD
	G	G	K	A	H	R	L	(SEQ ID NO:114)	1287.4	0.10
	G	G	K	A	N	R	L	(SEQ ID NO:115)	1113.9	0.01
	G	G	K	A	R	Q	L	(SEQ ID NO:116)	1021.9	0.13
	G	G	K	A	R	H	L	(SEQ ID NO:117)	939.3	UD
	G	G	K	A	R	N	L	(SEQ ID NO:118)	828.4	0.25
	G	G	K	A	dR	R	L	(SEQ ID NO:119)	494.4	0.06
	G	G	K	A	K	K	L	(SEQ ID NO:120)	77.9	UD
	G	G	K	A	H	K	L	(SEQ ID NO:121)	73.2	UD
	G	G	K	A	R	dR	L	(SEQ ID NO:122)	49.6	UD
	G	G	K	A	dR	dR	L	(SEQ ID NO:123)	16.5	UD--